Role of cytokines in the innate immune response to intracellular pathogens

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Interplay between innate and adaptive immunity

Intracellular pathogens live inside host cells, and survival is dependent on coexistence with the host. In their intracellular niche these pathogens are well shielded from the effector cells of the cellular immune system. However, microbial proteins are processed and presented, thus promoting activation of T lymphocytes. These T lymphocytes determine resistance, susceptibility, and often immunopathogenesis of intracellular infections. Pathogenic intracellular bacteria and parasites include different species of *Listeria*, *Mycobacteria*, *Salmonella*, *Chlamydia*, *Rickettsia*, *Trypanosoma*, and *Leishmania*. Although CD4+ lymphocytes are central to acquired resistance, an increasing amount of evidence is emerging showing crucial contributions by CD8+ T cells as well as unconventional T cells. These include CD4−, CD8− TCRαβ+ T cells recognising mycobacterial lipid antigens, T cells expressing the TCRγδ or natural killer T cells, which are thought to have a regulatory function in the early immune response. Facultative intracellular microbes favour mononuclear phagocytes as their biotope, but can survive in the extracellular environment. In contrast, obligate intracellular bacteria such as *Rickettsiae* and *Chlamydiae* must enter host cells, because their metabolism requires nutrients of the eukaryotic cell.

The different types of immune response fall into two categories: innate immune response and adaptive immune responses (table 1).

The major difference between innate and acquired immune responses is that the latter are highly specific for a particular pathogen. Moreover, although the innate immune response does not alter on repeated exposure to a given infectious agent, the adaptive response improves with each successive encounter with the same pathogen. Because innate immunity functions at times before adaptive immunity, its major role is likely to be to initiate defence early during primary infections. There is growing appreciation of the immunoregulatory role of the innate immune responses both in activating cellular constituents of innate immunity and in shaping downstream acquired responses. In addition to immediately activating effector functions of the innate cellular constituents, natural killer cells and phagocytes (for example, macrophages, dendritic cells) secrete soluble mediators that can modify cell trafficking to attract effector cells to sites of infections and concentrate T and B cells of the acquired immune system at sites of antigen presentation. The emerging picture is that in response to infection, immunocytes express a finely balanced and tightly regulated pattern of cytokines, which promote the most effective immunity against the infecting agent. As a result, innate immunity functions not only to protect the host from infection while slower adaptive immune responses are developing, but also to direct the qualitative and quantitative nature of adaptive immunity.

Table 1 Different types of immune response

Characteristics	*Innate immunity*	*Acquired immunity*
Specificity	Low	High
Diversity	Low	High
Specialisation	Low	High
Memory	No	Yes
Blood proteins	Complement	Antibodies
Cells	Phagocytes, NK cells	Lymphocytes

Growth of intracellular pathogens is restricted by several mechanisms acting in concert: (*a*) phagosome-lysosome fusion creates a hostile environment exposing the microbes to degrading lysosomal enzymes and a low pH; (*b*) macrophages restrict the availability of essential nutrients to the microbe—for example, the degradation of tryptophan has been associated with increased killing of *Chlamydia psittaci* and *Toxoplasma gondii*; (*c*) host cells produce highly reactive toxic molecules, particularly oxygen and nitrogen radicals, which are toxic for the microbe.

The following considerations aim at outlining the decisive role of cytokines and effector molecules which act early after microbial infection to shape a protective immune response. Firstly, the significance of cytokines in the innate immune response with focus on type I interferons (IFNα/β) will be highlighted using murine leishmaniasis as a model. Secondly, the differential induction of antibacterial activity against an important human pathogen, *Mycobacterium tuberculosis*, by cytokines and Toll-like receptors (TLR) will exemplify that the investigation of human cells is essential to gain detailed insight into the effector mechanisms of innate immunity.

Immunity to murine leishmaniasis

The hallmark of a protective immune response in mice against the protozoan parasite *Leishmania major* is the induction and expansion of CD4+ type 1 helper lymphocytes, which activate infected macrophages by the production of IFNγ for the killing of intracellular parasites.[1] IFNγ activates type 2 nitric oxide synthase (NOS2) in macrophages, leading to the production of reactive nitrogen intermediates that are toxic for intracellular *L major*.[2] The role of IFNγ in the control of infection

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with *L major* was established by studies showing that mice lacking IFNγ or the IFNγ receptor failed to resolve their cutaneous lesions.[3 4] The primary source of IFNγ during the innate response to *Leishmania* is the natural killer cell.[5 6] IFNγ production and cytolytic activity of NK cells is mediated by interleukin 12 (IL12) through the only recently recognised signalling function of NOS2.[7] Besides their function as signalling molecules, reactive nitrogen intermediates are major players in protection against intracellular *L major* by directly killing the pathogen.[4 8–10] On the other hand, *Leishmania* have evolved evasion mechanisms to survive within the hostile intracellular environment of macrophages, allowing persistence even in the presence of an intact cellular immune response of the host.[11] Potential survival strategies of the parasite include the suppression of NOS2,[12] induction of transforming growth factor β[13] and IL10, but not IL12,[14] and entry into NOS2 negative target cells as a haven.[15 16]

Evidence is accumulating that the outcome of murine leishmaniasis is critically dependent on the early events following the initial encounter of the parasite with its host cell, the macrophage. Keratinocytes, dendritic cells, natural killer cells, macrophages, CD4+ cells, cytolytic T cells, and granulocytes contribute to the composition of the local microenvironment by secreting chemokines (for example, MIP-1α, MCP-1), interleukins (for example, IL1, IL2, IL4, IL10, IL12), or, as described more recently, type I interferons (IFNα/β). The secretion of these molecules, the cross talk between them, and the cells responding to them are tightly intertwined. The resulting cytokine milieu governs whether naive T cells, which are either present in the lymph node or being recruited from the bloodstream, will develop into either protective Th1 cells or disease promoting Th2 cells.[17]

Type I interferons

Type I interferons are produced by a wide variety of cells, including macrophages, plasmacytoid monocytes, and dendritic cell precursors. The observation that type I interferons exert potent antiviral activity has led to their introduction into clinical practice as a first line treatment against chronic hepatitis B and hepatitis C.[18] In defence against bacteria and parasites IFNα/β modulates the synthesis of nitric oxide and inflammatory cytokines, macrophage activation by IFNγ, and the differentiation, activation, or proliferation of T helper cells. In addition to its regulatory functions IFNα/β contributes to protection against *Chlamydia*, *Toxoplasma*, *Leishmania*, *Trypanosoma*, *Listeria*, and *Mycobacteria*.[19]

In the mouse model of cutaneous leishmaniasis IFNα/β is already expressed at day one of infection in the skin lesion.[10] Functionally, IFNα/β plays a critical part in orchestrating the key events of the innate immune response to *L major*. Treatment of infected mice with anti-IFNα/β antibodies resulted in accelerated dissemination of the parasites, drastically reduced cytotoxic activity of natural killer cells, and elimination of the early peak in IFNγ production. The regulatory effects of IFNα/β are mediated by NOS2, as mice deficient in NOS2 failed to respond to treatment with purified IFNα/β.[10]

In vitro, simultaneous exposure of macrophages to IFNα/β and *L major* promastigotes induced NOS2.[20] The induction was critically dependent on the sequence of the stimuli. Pretreatment of macrophages with IFNα/β treated macrophages (one to two hours) failed to up regulate NOS2 upon exposure to *L major* parasites. The observation that macrophages become refractory to the costimulatory effect of *L major* after exposure to IFNα/β might contribute to the limited expression of NOS2 during the early phase of infection with *L major*.[13] Thus the early expression of IFNα/β after infection with *L major* allows the host organism to express small amounts of NOS2 that are required for the activation of NK cells, but also helps the parasite to survive by limiting the number of NOS2 positive macrophages.

Immunity in human tuberculosis

Successful elimination of the intracellular pathogen *M tuberculosis* depends mainly on the efficient interaction between infected macrophages and antigen-specific T cells. The crucial contribution of T cells is underlined by the clinical observation that patients with impaired T cell function (for example, old age,[21] corticosteroids,[22] or AIDS[23 24]) are at increased risk of developing clinically manifest tuberculosis. In contrast, people with defective humoral immunity, such as those with sickle cell disease and multiple myeloma, show no increased predisposition to tuberculosis. Recent advances in the characterisation of the protective immune response to *Mycobacteria* have highlighted the central role of phenotypically and functionally distinct subsets of T cells. These T cell subsets contribute to host defence not only by the secretion of macrophage activating cytokines such as tumour necrosis factor (TNF) or IFNγ[25] but also by lysing the infected host cell.[26 27] Besides releasing intracellular pathogens, which can then be taken up and killed by newly recruited macrophages,[28] it has been shown that CD8+ T cells release granulysin, which directly kills the pathogen.[29]

Our understanding about immunity against tuberculosis is mainly based on experiments performed in mice.[30] The significance of these findings for the immunopathogenesis of human tuberculosis is under debate. The cellular infiltrate in the murine lung does not reflect the classical granulomas observed in human tuberculosis. More importantly, mice generally die of disseminated tuberculosis after four to six months depending on the genetic background, whereas humans are much less susceptible, and only a minority of infected patients develop disease.

Toll-like receptors (TLR)

TLR are expressed on the cell surface of mammalian cells, particularly phagocytes, and

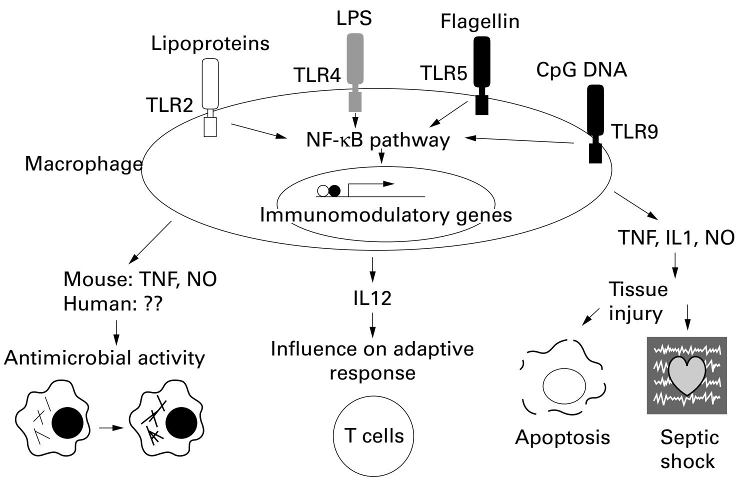

Figure 1 Toll-like receptors and host defence.

represent highly conserved homologues to the *Drosophila* Toll system.[31] Microbial ligands, including lipopolysaccharide and bacterial lipoproteins, bind toTLR, facilitating NF-κB translocation to the nucleus, resulting in the transcription of genes with immunoregulatory function, including cytokines and costimulatory molecules (fig 1).[32–34] It has been shown that TLR2 activation leads to killing of intracellular *M tuberculosis* in both mouse and human macrophages.[35] In mouse macrophages, bacterial lipoprotein activation of TLR2 leads to an NO dependent killing of intracellular tubercle bacilli. In human monocytes and alveolar macrophages, bacterial lipoproteins similarly activated TLR2 to kill intracellular *M tuberculosis*, but by an antimicrobial pathway that is NO independent. This suggests that similar antibacterial effector pathways are active in mice and humans, but the executing molecules are apparently distinct. This finding was extended by use of a potent stimulus known to induce NO in mouse macrophages—namely, the combination of TNF and IFNγ .[36 37] In murine peritoneal macrophages, TNF plus IFNγ induced the production of NO and reduced the viability of intracellular *M tuberculosis*. In contrast, in human macrophages, the combination of TNF and IFNγ neither induced detectable NO production nor exerted any antimicrobial effect, but did induce IL12 release.[35] These results indicate that the mouse and human TLR pathway has similarly retained the ability to activate direct antimicrobial effector mechanisms, even in the absence of immune T cells. A striking observation was that TLR activation led to distinct pathways of antimicrobial activity in mice and humans. Whereas in mice, TLR activation leads to an NO dependent antimicrobial pathway, in humans the TLR activated antimicrobial pathway is NO independent.

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